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Comparison of bacterial communities in faeces of beef cattle fed diets containing corn and wet distillers' grain with solubles

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Abstract

Aim: The mammalian intestinal microflora has been shown to impact host physiology. In cattle, intestinal bacteria are also associated with faecal contamination of environmental sources and human illness via foodborne pathogens. Use of wet distillers' grains with solubles (WDGS) in cattle feed creates a gastrointestinal environment where some bacterial species are enriched. Here, we examine if a diet containing 40% WDGS results in fundamentally different microbial community structures.

Methods and Results: The 20 002 16S r-RNA gene sequences from 20 beef cattle were analysed using Sanger sequencing methods. At the genus level, *Prevotella* (Gram negative) and *Anaerobacter* (Gram positive) were the most frequently occurring bacteria in our beef cattle faecal samples. Diet-associated differences in prevalence were noted for *Prevotella* but not *Anaerobacter*.

Conclusions: Diet affects community structure. Faecal communities of co-housed beef cattle are not identical.

Significance and Impact of the Study: It is known that a diet of 40% corn-based WDGS increases the generic *Escherichia coli* in the faeces and enriches *E. coli* O157:H7. The results from the current study suggest that in addition to previously observed changes in *E. coli*, the entire bacterial community structure is different for animals fed 40% corn-based WDGS compared to a traditional corn-finishing diet.

Introduction

Cattle gastrointestinal communities impact animal health and nutrient uptake. Defining these habitats is of interest in bacterial source tracking applications because of potential for livestock contamination of ground and surface waters, and in human health because of the potential for cattle faeces to serve as a vector for foodborne pathogens. The bovine faecal microbiota has been shown to be impacted by diets containing distillers' grains (Jacob *et al.* 2008; Callaway *et al.* 2010; Muegge *et al.* 2011; Shanks *et al.* 2011; Rice *et al.* 2012). Distillers' grain is a by-product of ethanol production and is a good source of protein and energy for cattle (Spiehs *et al.* 2002; Schingoethe *et al.* 2009). It is made from a variety of grains, including corn, wheat and sorghum. Both wet and dried distillers' grains are commonly used as cattle feed. The specific differences observed in the bovine faecal microflora in response to feeding of distillers' grains depend on the amount and formulation of distillers' grains being fed.

In a study conducted in the Southwestern United States, the feeding of dried distillers' grains to four animals in Texas resulted in an increase in faecal *Acinetobacter* compared to controls (Callaway *et al.* 2010). In a second

study based in the United States, the feeding of a processed grain diet containing steam-flaked, dry-rolled and/or distillers' grain corn to ten animals in Colorado revealed diet-associated changes in the faecal microflora for the family Ruminococcaceae and the genus *Prevotella* compared to control animals from Nebraska, Georgia and Ohio fed forage or unprocessed grains (Shanks *et al.* 2011). The feeding of a 10% corn- or 5–15% sorghum-based distillers' grain diet to 200 animals on five diets results in phylum-level changes in the Synergistetes, WS3, Actinobacteria and Spirochaetes (Rice *et al.* 2012). Feeding of a 40% corn-based wet distillers' grain with solubles (WDGS) diet for beef feedlot cattle resulted in shifts in the pH, volatile fatty acids and L-lactate of the animal faeces. Changes in the number of both faecal indicator *Escherichia coli* and pathogenic *E. coli* were also noted (Wells *et al.* 2009, 2011).

Culture-based studies are excellent for measuring the prevalence of specific target bacteria, but do not provide any information on the broader microbial community composition or structure. Our aim was to characterize the faecal microflora in animals on control and distillers' grain diets and to quantitatively assess whether the underlying faecal bacterial community structure remains the same or changes in response to the 40% WDGS diet.

Materials and methods

All animal procedures were reviewed and approved by the US Meat Animal Research Center Animal Care and Use Committee, and rations were formulated to meet or exceed recommendations of the National Research Council (1996). Animals for this study were chosen from a larger set of 608 feedlot steers and were fed a corn-based finishing diet, or a 40% WDGS diet. The WDGS diet was formulated for a final concentration of 40% WDGS (Wells *et al.* 2009). Rectal-grab faeces were collected from each of 20 beef steers after they had been on the finishing diet for 150 days. We used 16S rRNA gene sequencing to examine the bacterial communities of 20 feedlot beef cattle fed standard corn-based or 40% corn-based WDGS finishing diets. Experiments were specifically designed to limit factors that could contribute to observed differences in bacterial communities such as animal age, gender, breed and macroecologic factors such as temperature and rainfall. A total of 10 libraries were made from 20 animals. Five libraries were made from animals on the corn diet: four libraries from each of four individual animals, and one pooled library from an additional six animals. Five libraries were made from animals on the WDGS diet: four libraries from each of four individual animals, and one pooled library from an additional six animals (Table S1). Total DNA was isolated from each sample using a direct lysis procedure, followed by TOPO TA cloning and sequencing using an ABI 3700, as previously described (Smith *et al.* 2000; Durso *et al.* 2010). Rarefaction curves were used to determine the extent of sequence coverage. Sequences were screened for quality using Phred scores and for chimeras using the Mothur suite of tools (Schloss *et al.* 2009). All low-quality reads and suspected chimeric sequences were excluded from analysis. Mothur tools were used to assign sequences to operational taxonomic units (OTUs) based on DNA sequence similarity and to calculate the Chao I abundance coverage estimator and the Shannon diversity index (Table S2). Mothur tools were used for sequence processing, OTU-based comparisons and OTU-based hypothesis testing approaches (Schloss *et al.* 2009). For Libshuff analysis, the critical threshold for deciding if a result was statistically significant was calculated as 0·025 (0·05 divided by the two diets analysed). In addition to OTU-based analysis, sequences were assigned a taxonomy using the naive Bayesian rRNA classifier associated with the Ribosomal Database Project (Cole *et al.* 2003). After assigning taxonomies, comparisons were made both manually, and using the RDP 'library compare' tool, which uses a two-population proportions test for all groups in this study. Heat maps based on RDP-assigned taxonomies were constructed using the double hierarchal cluster analysis in NCSS2007 software (NCSS, Kaysville, UT, USA). Pooled data sets were excluded from heat map analysis. Abundance of each OTU in each sample set was calculated as a percentage of total reads in sample and compared across treatments using GLM procedures in SAS (SAS Institute, Cary, NC, USA) with sample as experimental unit. Effects of dietary treatment were determined for individual samples alone, and individual and pooled samples combined, and are noted in the results. Differences in means were considered significant when P values were $< 0{\cdot}05$.

Results

There were 20 002 16S rRNA gene clones sequenced that passed the minimum quality filters: 10 330 from corn libraries ($n = 5$ corn libraries total: four individual animals, and one pool of six animals) and 9672 from WDGS libraries ($n = 5$ WDGS libraries total: four individual animals, and one pool of six animals). Average read length was 730 bases. The top ten most frequently occurring OTUs, based on number of sequences assigned, are listed in Table S3. Using a 97% sequence similarity cut-off, there were 14 591 OTUs, only 660 (4·5%) of which contained sequences from both corn and WDGS samples. There were 7085 OTUs containing sequences exclusively from corn libraries, and 6846 OTUs containing sequences exclusively from WDGS libraries. When the sequences

from the pooled libraries were removed from the analysis and only data from the eight individual animals were examined, there were 11 424 OTUs assigned using a 97% DNA sequence similarity cut-off (n = 6147 in the corn libraries, n = 5723 in the WDGS libraries). Of these, 446 (4%) were found both in the corn and the WDGS libraries.

Libshuff analysis was run to test the hypothesis that the fundamental bacterial community structure is different in faeces from beef feedlot cattle fed corn- or WDGS-based diets. The initial analysis was performed on the two pooled libraries (six animals from each diet) and indicated that the bacterial community structure in the two libraries was distinct ($P < 0{\cdot}0001$). Additional eight libraries were made from each of the eight individual animals – four animals on each diet. Libshuff analysis on the individual libraries based on diet (corn and WDGS) confirms the results obtained with the two pooled libraries ($P < 0{\cdot}0001$; Table S4). SAS analysis of the OTU distribution allowed us to identify OTUs that might be contributing to these

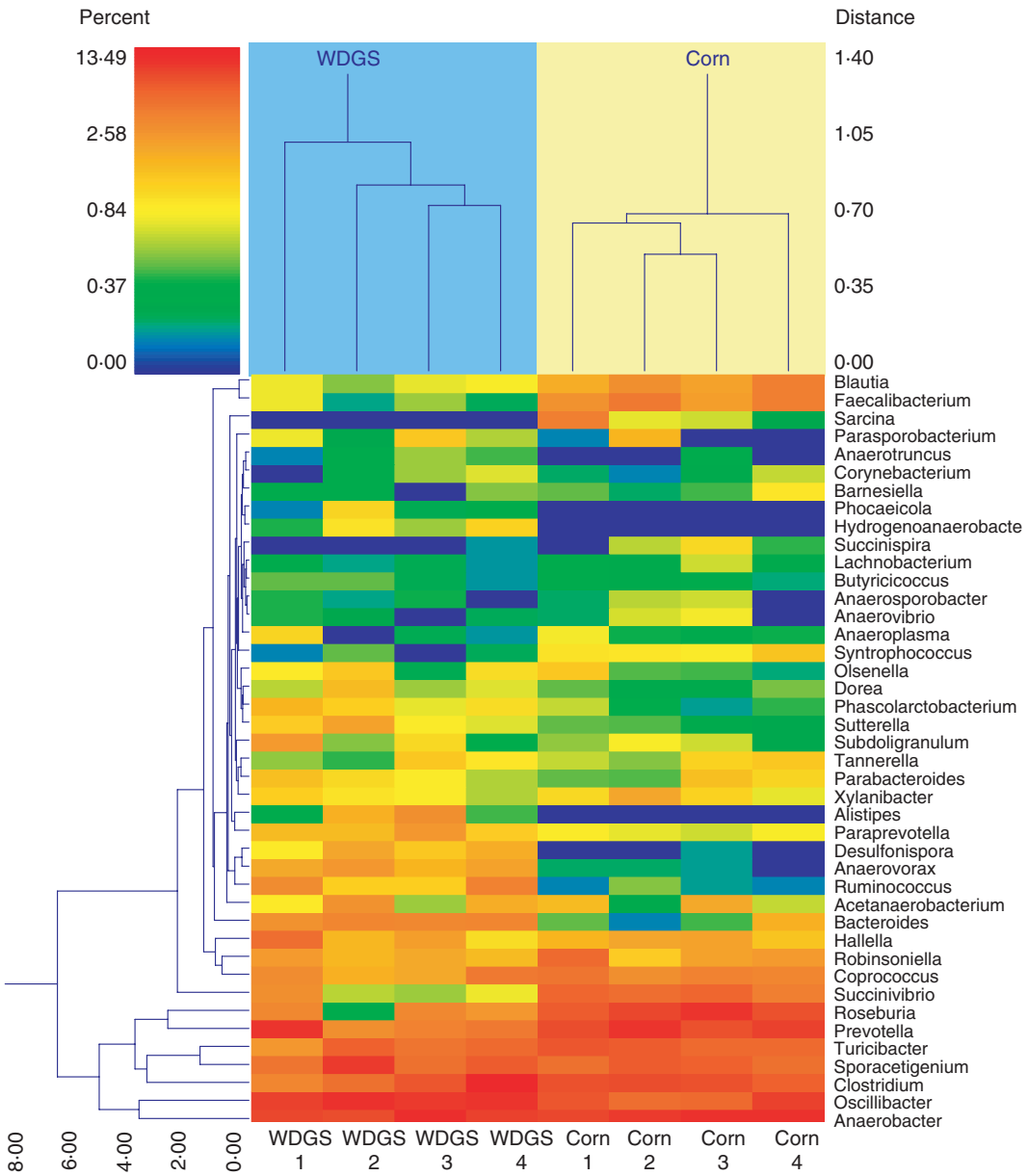

Figure 1 Heat map of top RDP-assigned genera, by abundance. Analysis is performed on eight libraries made from eight individual animals (n = 4 animals fed a diet of 40% wet distillers' grains with solubles (WDGS), and n = 4 animals fed a control corn diet). Per cent indicates the per cent of clones from each library assigned to a particular taxonomy.

differences. Significant differences in relative abundance of OTUs, as determined by the SAS analysis, are listed in (Table S5). The SAS analysis was performed both for the individual animal data and for the combined individual animal and pooled animal data, using a 97% DNA sequence similarity cut-off. In the individual animal library data set, 27 OTUs were identified that contained at least 10 sequences that displayed a statistically significant difference in distribution between the corn and WDGS libraries. In the combined individual and pooled library data set, 14 OTUs were identified.

All 16S sequences were assigned taxonomies using the RDP Classify program. Ten phyla were identified in the sequences from our samples. Averages from animals on each diet are almost identical, although even at this lowest level of taxonomic resolution there are animal-to-animal differences in the distribution of clones assigned to each phylum for each of the individual animal libraries. At the genus level, *Prevotella* (Gram negative) and *Anaerobacter* (Gram positive) were the most frequently occurring bacteria in our beef cattle faecal samples, with 14 and 13% of all clones being assigned, respectively, to these two genera. *Prevotella* assignments represented 8·6% of the clones in the corn libraries, and 3·8% of the clones in the WDGS libraries. *Anaerobacter* assignments represented 6·2 and 5·5% of the corn and WDGS libraries, respectively. Genus-level RDP assignments for the eight individual animal libraries are displayed by diet as a heat map in Fig. 1. The top 42 genera are listed, representing 97% of the clones assigned at the genus level. Genera that were consistently more abundant in the animals fed the corn diet include *Blautia*, *Faecalibacterium* and *Sarcina*. Genera that were consistently more abundant in the animals fed the WDGS diet include *Ruminococcus*, *Desulfonispora*, *Alistipes*, *Hydrogeoanaerobacterium* and *Phocaeicola*.

Discussion

Results of this study indicate that the overall bacterial community structure is distinct in faeces from beef cattle fed corn compared to beef cattle fed a 40% corn-based WDGS diet. Although many of the most frequently occurring genera reported as part of this study have also been reported with feeding dried corn- and wet sorghum-based distillers' grains, some WDGS-specific OTUs were observed, primarily for *Oscillospira* and *Eubacterium*. Heat map analysis using taxonomic assignments highlights *Blautia*, *Faecalibacterium* and *Sarcina* as genera that are over-represented in the corn diets, and *Ruminococcus*, *Desulfonispora*, *Alistipes*, *Hydrogeoanaerobacterium* and *Phocaeicola* as over-represented in the WDGS diets. Of these, only *Ruminococcus* and *Hydrogeoanaerobacterium* were found to be diet associated in the dried corn- or wet sorghum-based distillers' grains studies (Callaway *et al.* 2010; Shanks *et al.* 2011; Rice *et al.* 2012). Results from the current study confirm that, even at the phylum level, the distribution of OTUs was not identical for either corn- or WDGS-fed animals, despite the fact that the animals all came from a single population (Table S6).

One trend that emerges from examination of the results of this and previous studies is that the *Prevotella* are consistently identified as frequently occurring members of the bovine faecal flora and that members of the *Prevotella* appear to be correlated, consistently and across multiple studies, to changes in feeding of multiple sources and amounts of distillers' grains compared to corn (Callaway *et al.* 2010; Shanks *et al.* 2011). The *Prevotella* also appear to be correlated with dietary changes in other mammals, including humans (Wu *et al.* 2011). In humans, three basic groups of intestinal microbial consortia, called enterotypes, have been described (Arumugam *et al.* 2011). An examination of the dietary patterns linked with the different enterotypes noted a positive correlation between *Prevotella* and diets rich in carbohydrates, as well as a correlation between *Bacteroides* and diets high in fat and protein (Wu *et al.* 2011). While there are significant differences between the diet and physiology of humans and cattle, it is interesting to note that our results also show diet-related shifts in *Prevotella* and *Bacteroides*. Other genera of interest in this study are *Sporacetigenium* and *Anaerovorax*, both of which are over-represented in the faeces of animals fed the WDGS diet. *Sporacetigenium* has not been well characterized, but one strain from an anaerobic digester was reported to ferment a variety of pentose sugars (Chen *et al.* 2006). The WDGS is expected to be rich in pentoses owing to the greater level of hemicellulose in the WDGS component. *Anaerovorax* has been characterized as a putrescine-fermenting bacterium (Matthies *et al.* 2000). Putrescines typically arise from amino acids and proteins, which are expected to be higher in the WDGS diet.

In conclusion, our results indicate that the faecal bacterial community structure from animals fed 40% corn-based WDGS is distinct from that of animals fed corn. This suggests that faecal bacteria may face different kinds of selective pressures in faeces from animals fed 40% corn-based WDGS diet compared to faeces from animals fed traditional corn-finishing diets.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Animal and Library Information for individual and pooled libraries evaluated to determine faecal bacterial community structure of beef cattle fed diets of corn or 40% wet distillers' grains with solubles (WDGS). A total of 10 libraries were made from 20 animals.

Table S2 Richness and diversity statistics for faecal bacterial libraries from beef cattle fed corn or 40% wet distillers' grains with solubles (WDGS) diets. Calculated at 97, 95 and 90% DNA sequence similarity. Chao I indicator includes Lower Confidence Interval (LCI) and Higher Confidence Interval (HCI).

Table S3 Top 10 most frequently occurring OTUs in faeces of cattle fed corn or 40% wet distillers' grains with solubles (WDGS).

Table S4 Libshuff values for pooled and individual library comparisons for beef cattle fed diets of corn or 40% wet distillers' grains with solubles (WDGS). The initial analysis was performed on two pooled libraries (six animals from each diet), and indicated that the bacterial community structure in the two libraries was distinct ($P < 0.0001$). An additional eight libraries were made from each of eight individual animals – four animals on each diet. Libshuff analysis on the individual libraries based on diet (corn and WDGS) confirm the results obtained with the two pooled libraries ($P < 0.0001$).

Table S5 OTUs with >10 sequences displaying statistically significant difference in distribution of clones based on diet ($P \leq 0.05$). Analysis is based on DNA from 10 animals fed a corn diet, and 10 animals fed a diet of 40% wet distillers' grains with solubles (WDGS).

Table S6 Phylum level distributions of fecal library clones from beef cattle fed corn or 40% wet distillers' grains with solubles (WDGS). A total of 10 libraries were made from 20 separate animals.

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